

# Seasonal Timing of Diapause Induction Limits the Effective Range of *Diorhabda elongata deserticola* (Coleoptera: Chrysomelidae) as a Biological Control Agent for Tamarisk (*Tamarix* spp.)

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Environ. Entomol. 36(1): 15–25 (2007)

**ABSTRACT** The leaf beetle *Diorhabda elongata* Brullé subspecies *deserticola* Chen, collected in northwestern China, has been released in the western United States to control tamarisk (*Tamarix* spp.). While beetle establishment and saltcedar defoliation have been noted at northern study sites, this species has not established at latitudes south of the 38th parallel. Critical daylength for diapause induction was measured in the laboratory and ranged between 14 h 50 min to 15 h 08 min, depending on temperature, and adults were shown to cease reproduction and enter diapause at daylengths of 14 h 30 min or less. Critical daylength in the field was measured at  $\approx 14$  h 39 min and occurred 13 d before 50% of the population reached diapause. South of 36°20' N, the longest days of the year are shorter than 14 h 39 min, making the beetles univoltine in the southern United States. North of 36°20' N, a window of reproductive activity opens 13 d after the critical daylength is reached in the spring and closes 13 d after it is passed in the summer, allowing at least a partial second summer generation. It is predicted that south of the 38th parallel, premature diapause will increase mortality and disrupt synchrony between the life cycle of the beetle and host plant availability. This could hinder establishment and help explain the failure of this population south of the 38th parallel, providing a rationale for testing other populations of *D. elongata* in the southern range of *Tamarix* in North America.

**KEY WORDS** *Diorhabda elongata*, photoperiod, thermoperiod, tamarisk, biocontrol

Tamarisks (*Tamarix* spp.) are exotic invasive weeds causing extensive economic and ecological damage across western North America (Dudley et al. 2000, Zavaleta 2000, Shafroth et al. 2005). A large scale biological control program was initiated in 1987 and is now well underway with the input and participation of researchers and stakeholders from >40 government agencies, universities, and private organizations in the United States and abroad (DeLoach et al. 2003). Overseas exploration has yielded several promising biocontrol agents, but the only one thus far approved for field release is the chrysomelid leaf beetle, *Diorhabda elongata* Brullé subspecies *deserticola* Chen (DeLoach et al. 2003). The insects were first released into cages in 1999 and into the open at selected sites in 2001. At some sites, beetle populations have expanded rapidly and have defoliated hundreds to thousands of acres of *Tamarix* in 5 yr, whereas at other sites, including sites

in regions with the most serious tamarisk infestations, the insects have failed to establish (DeLoach et al. 2004). The potential benefits of the tamarisk biocontrol program have become clear with the dramatic successes at some field sites. However, to realize the full potential of *D. elongata* throughout the range of tamarisk in North America, the reasons for failure to establish must be understood.

Biocontrol agents may fail because they are not adapted to the new environments into which they are released (McClay and Hughes 1995) or are not adapted to the target genotype against which they are released (Lym et al. 1996, Sobhian et al. 2003) or they may encounter novel predators and parasitoids. Failure of *D. elongata* may be the result of one or more of these problems. Predation has been noted at most of the sites, and ant predation was shown to have a major impact on the survival of beetle larvae at one of the failed sites (Herrera 2003). Target *Tamarix* may present novel genotypes because extensive hybridization has been shown between some of the invasive species (Gaskin and Schaal 2002). In addition, a pattern was noted in which beetles at lower latitude sites (38° N or lower) have not established, whereas at higher latitudes, populations have established and are expanding (Lewis et al. 2003). In the early summer at the Temple, TX, field site (31°06' N), adult beetles were found in an apparent state of reproductive dia-

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pause, and it was suggested that a short-day induced premature diapause could be hindering establishment of the leaf beetle in Texas (Lewis et al. 2003). Further information on *D. elongata* diapause, and the cues that trigger it, were required to better understand and remedy the problem.

*Diorhabda elongata* overwinters in reproductive diapause and emerges in the spring when *Tamarix* foliage becomes available (Li et al. 2000, Lewis et al. 2003). By mid-winter, diapause is no longer maintained by short day photoperiods and beetles are prepared to resume development when temperatures become favorable (D.B., unpublished data), as is the case with many insect species (Tauber and Tauber 1970, Tauber et al. 1986). The overwintering generation is reproductive in the spring and always produces a first summer generation that emerges as adults by late spring or early summer.

The first summer generation may enter diapause and become the overwintering generation, as seen at the Temple site, or may become reproductive and produce a second summer generation as noted at more northern sites in the United States (Lewis et al. 2003). In the native range of this beetle, both the first and second summer generations are reproductive, and the third summer generation becomes the overwintering generation (Li et al. 2000). From these observations, it is apparent that *D. elongata* has a facultative diapause and that environmental cues may dictate the timing of diapause induction and the number of generations per season. Photoperiod is one likely cue for diapause induction in this beetle because most temperate zone insects respond to photoperiod (Beck 1980, Saunders 2002).

Critical daylength is a genetically determined property of a population and is defined as the daylength at which 50% of the individuals in a population enter diapause (Beck 1980, Saunders 2002). Many temperate zone species continue development when days are long and enter diapause when days are short, with the critical daylength falling between these (Beck 1980, Tauber et al. 1986). Critical daylength varies depending on the species being considered and the origins of the population being considered (Danilevsky et al. 1970, Bradshaw 1976, Masaki 1999). In the case of *D. elongata*, the occurrence of only one generation per year suggested that early summer daylengths at the Temple field site are shorter than the critical daylength. The occurrence of multiple generations at more northern test sites, as well as at the place of origin in China, suggested that at these sites early summer daylengths exceed the critical daylength for diapause induction, allowing reproduction and multiple generations during a season (Lewis et al. 2003). Daylengths at the latitude of origin for this population (44°10' N) reach ≈15 h 30 min, whereas at the test site in Temple, TX (31°06' N), daylengths only reach 14 h 10 min (Lewis et al. 2003). If the critical daylength for diapause induction falls between 15 h 30 min and 14 h 10 min, the majority of first-generation adults will enter diapause at the Temple field site, whereas first-generation adults would be reproductive in northern

China and field sites in the northern United States. This could explain why the beetles are univoltine at the Temple site and may explain the failure of beetles to establish in the southern United States.

The following study was initiated because the critical daylength and the factors that influence critical daylength, such as temperature, are important for predicting life cycle timing (Tauber et al. 1986, Beck, 1980), and thus the potential success of *D. elongata* as a biocontrol agent. This study defines critical daylength for diapause induction and uses the value to predict seasonal timing of reproduction in the field. The relationship between latitude and reproductive activity is described for *D. elongata*, and from this, the geographic limits for establishment of this biocontrol agent are estimated.

## Materials and Methods

**Insects and Field Sites.** Insects (*D. e. deserticola*) used in laboratory studies were from a continuous stock culture, maintained at 25°C and a photoperiod of 16:8 (L:D) h. They were established and periodically replenished using field-collected material from study sites in the western United States, where beetles have been in cages since 1999 and released into the open in 2001 (Lewis et al. 2003). All insects used in these studies originated near the town of Fukang in the Xinjiang Province of China (44°10' N, 87°59' E) (DeLoach et al. 2003). Culturing procedures used to maintain the colonies have been described elsewhere (Bean et al. 2007).

Field sites used in this study were selected in a wide-ranging study to test the safety and efficacy of *D. elongata* in tamarisk biocontrol (DeLoach et al. 2003). In Nevada, there were two sites: one located 17 km SE of Lovelock (40°02' N, 118°31' W) and known as the Lovelock site, and one located on the Walker River, north of Walker Lake (38°53' N, 118°45' W) known as the Walker site. In California, there was a single site located in the Owens Valley between Independence and Big Pine on the Tinemaha Reservoir (37°05' N, 118°14' W) known as the Tinemaha site. In Colorado, there was a single site located near Pueblo (38°16' N, 104°37' W) and known as the Pueblo site. In Wyoming, there was a single site located 18 km east of Lovell (44°50' N, 108°23' W) known as the Lovell site. In Temple, TX (31°06' N, 97°22' W), beetles were introduced but failed to survive, even in cages (Lewis et al. 2003), and this is known as the Temple site.

**Diapause Induction Studies.** Insects taken from the stock culture were started as eggs or first-instar larvae under conditions of constant temperature and photoperiod in growth chambers or incubators (Hotpack, Warminster, PA or model I30BLL; Percival, Perry, IA). The sensitive period for diapause induction extends into the adult stage (Bean et al. 2007), so that insects reared from eggs or first instars are exposed to the test photoperiods during the sensitive portion of their lifecycle. Larvae were mass reared in groups of at least 20, transferred to sand for pupation, and when adults emerged, they were paired and given fresh

foliage. The number of pairs tested at each photoperiod and temperature ranged from 25 to 84, with most conditions having at least 35 pairs. Paired adults were kept under experimental conditions for at least 15 d but usually 20 d after emergence, and if no oviposition occurred, the insects were scored as diapause. It had been shown that under conditions that promote continuous development, 100% of females oviposit within the first 8 d after adult emergence (Bean et al. 2007). In some experiments, females that had not oviposited were dissected after 20 d, and no reproductive development was found, thus confirming the diapause state. In some instances, males were also dissected, and it was noted that their reproductive condition was equivalent to the condition of females under the same treatments.

Critical daylength was estimated using logistic regression analysis (JMP, SAS Institute 2000). Reproductive status was the binary response, dependent variable (0 for diapause, 1 for reproductive) plotted against hours of light. The  $P$  value for  $\chi^2$ , testing regression model significance, was  $<0.0001$  in all cases. Inverse prediction was used to calculate the daylength at which there is a 0.50 probability of diapause (critical daylength) plus 95% CL. Critical daylength was also determined by a direct estimation in which there is a proportional allotment of daylength between the two measurements on either side of the 50% diapause point (Kurota and Shimada 2003), and the results were nearly identical to those obtained using logistic regression.

**Thermoperiod Experiments.** A thermoperiod was tested that had an average temperature of 25°C and fluctuated between 9 h at 34°C and 9 h at 16°C. Chambers made a continuous transition between the high and low temperatures over a 3-h period either by programming a continuous transition or by programming a series of 1.8°C steps, depending on the capabilities of the chamber. The temperatures almost exactly match the average temperature and the average high and low temperatures recorded at the Tinemaha site on 21 June. Photoperiod was superimposed onto the thermoperiod such that the scotophase exactly coincided with the cryophase at the 15:9 (L:D)-h photoperiod. At photoperiods of 14.5:9.5 and 14:10 (L:D) h, the scotophase began after the initiation of the cooling and ended at the initiation of the warming.

**Photoperiod Switch Experiments.** Insects were reared to adulthood at 16:8 (L:D) h, 25°C and divided into groups of 30 pairs, and groups were switched from 16:8 (L:D) h to the experimental photoperiods after all pairs had begun to lay eggs, day 9 after adult emergence. The experimental photoperiods were 12:12, 13:11, 14:10, 14.5:9.5 and 15:9, with controls remaining at 16:8 (L:D) h, and all treatments were at 25°C. Pairs were monitored daily for oviposition, and if a pair failed to lay eggs for 10 consecutive d, they were considered to be in diapause from the first day without oviposition. Beetles maintained at 16:8 (L:D) h laid eggs either daily or every other day. Dissection of some adults that had ceased oviposition showed de-

velopment of fat body and regression of ovaries, indicative of diapause (Bean et al. 2007).

**Diapause in Field-Collected Insects.** During the spring and summer of 2001 and 2002, newly emerged adult beetles were collected from cages at Tinemaha and were placed into mesh sleeves on live *Tamarix ramosissima* plants. Similarly, newly emerged adults were collected from established field populations in defoliated areas at Lovelock and Walker during spring and summer of 2004 and divided into three to six replicate mesh sleeves. Insects were held for a minimum of 5 d in the sleeves to allow the completion of reproductive development or the development of the fat body as an indicator of diapause. Holding insects in sleeves also prevented the migration of diapause-destined individuals to the leaf litter (Bean et al. 2007). Adults were also collected at regular intervals during the summer of 2002 at Lovell by David Kazmer (USDA-ARS NPARRL, Sidney, MT) and during the summer of 2003 at Pueblo by Debra Eberts (USBR, Denver, CO). At both of these sites, beetles were well established outside of the cages. Beetles from the Lovell and Pueblo sites were collected directly from the field or from field cages and preserved in 50% ethanol/water, 1:1 vol:vol. Because they were taken directly from the field, some of the collections contained up to 80% newly emerged adults, which had neither well-developed fat body nor developed reproductive systems, and these were not factored into the calculation of diapause percentage.

Determination of developmental status of field collected insects was made by dissection and scoring the reproductive system and fat body, as described elsewhere (Bean et al. 2007). Beetles collected from Tinemaha, Walker, and Lovelock were either chilled or frozen and dissected under saline (200 mM NaCl in distilled water). Beetles from the Lovell and Pueblo sites, stored in 50% ethanol, were dissected under saline. Although storage in 50% ethanol changed the color and texture of the fat body and reproductive systems, they could still be scored for developmental status. Dissected adults were placed into one of three different categories: diapause, reproductive, and newly emerged. The criteria used to separate these are described in detail elsewhere (Bean et al. 2007), but essentially, diapause individuals had well-developed fat body and little reproductive system development. Reproductive individuals had well-developed reproductive systems and little fat body, whereas newly emerged insects had little fat body along with undeveloped reproductive systems. Insects that had been previously reproductive but were entering diapause had reproductive systems in a state of resorption (regressing) and had abundant fat body.

To determine the dates of 50% diapause for field populations, logistic regression analysis was used with developmental state as the dependent, binary variable (0 for diapause, 1 for reproductive) plotted against date of collection (JMP, SAS Institute 2000). The  $P$  value for  $\chi^2$ , testing regression model significance was  $<0.0001$  in all cases. Inverse prediction was

**Table 1.** Critical daylength for diapause induction of *D. elongata* measured at five different temperatures and one thermoperiod

Temperature (°C)	Critical daylength <sup>b</sup> (h)	95% confidence interval <sup>c</sup>
22	15.06	14.97, 15.18
25	15.13	15.06, 15.23
28	14.91	14.81, 15.01
31	14.83	14.73, 14.93
34	14.23	14.10, 14.43
34–16 thermoperiod <sup>a</sup>	14.88	14.78, 15.01

<sup>a</sup> Cycling between 34 and 16°C with an average of 25°C.

<sup>b</sup> Critical daylengths were calculated by logistic regression.

<sup>c</sup> The 95% confidence intervals were calculated for the 50% diapause point.

used to calculate the date at which there is a 0.50 probability of diapause, plus 95% CL.

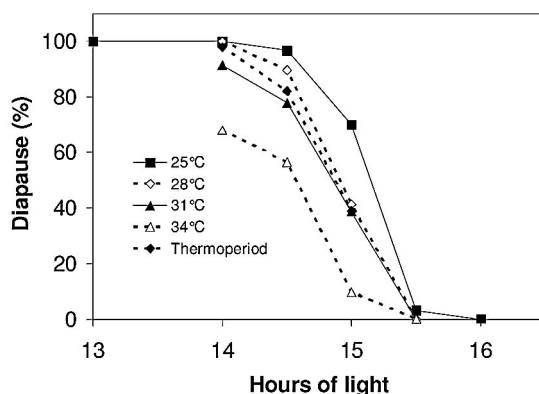
Throughout this study, daylengths were determined using sunrise/sunset tables provided by the U.S. Naval Observatory (<http://aa.usno.navy.mil>), and temperature data were obtained from the National Climatic Data Center, U.S. Department of Commerce ([www.ncdc.noaa.gov](http://www.ncdc.noaa.gov)).

## Results

**Critical Daylength for Diapause Induction.** The critical daylength for diapause induction ranged between 15 h 08 min and 14 h 50 min at temperatures from 22 to 31°C, and at 34°C, the critical daylength was calculated at 14 h 14 min using logistic regression, even though >50% of the insects entered diapause at 14 h 30 min (Table 1). It seems that a portion of the population fails to enter diapause at high temperatures under any daylength, even as low as 10 h (data not shown). Constant high temperatures do not reflect field conditions under most circumstances, so a thermoperiod of 34°C/16°C was tested, matching the average daily temperature fluctuation at Tinemaha on 21 June. Critical daylength under this thermoperiod was 14 h 53 min, nearly the same as the critical daylength measured at a constant 28°C (Table 1).

Diapause induction curves for *D. elongata* have a steep slope, going from nearly 100% diapause to nearly 100% reproductive with a 1-h change in daylength (Fig. 1). At a daylength of 15 h 30 min, almost all insects were reproductive, whereas at 14 h, almost all insects enter diapause at temperatures of 31°C and below.

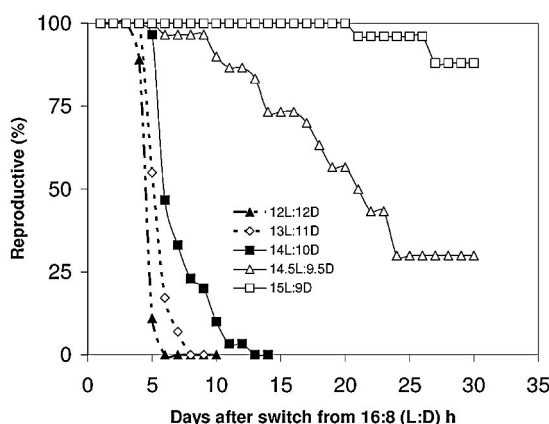
**Reproductive Adults Enter Diapause Under Short Days.** When reproductive adults were shifted from a photoperiod that enables continuous reproduction, 16:8 (L:D) h, to photoperiods with shorter daylengths, the insects ceased reproduction and switched to diapause (Fig. 2). Oviposition ceased rapidly, within 5–7 d under daylengths of 12 or 13 h, consistent with previous results (Bean et al. 2007). Oviposition persisted longer at daylengths between 14 and 15 h. At 14 h, the majority of females ceased oviposition after 6 d, but ≈30% continued to oviposit for an additional 3–4 d. At 14.5 h of light, reproduction continued for an extended time in most females; 25% of the insects



**Fig. 1.** Induction of reproductive diapause under different temperatures and daylengths. Each point represents 25–84 pairs of insects. The induction curve for 22°C nearly overlaps with the 25°C curve so it was not shown. Insects were reared under constant temperature and photoperiod from egg or first instar until adulthood and were paired after emergence from pupal sand cases. The thermoperiod was 34/16°C, with an average of 25°C.

remained reproductive after 30 d. At 15 h of daylength, most insects remain reproductive for at least 30 d. These results indicate a critical daylength of ≈14.5 h light for diapause induction in previously reproductive adults. At daylengths <14.5 h, reproductive adults switch rapidly to diapause. At daylengths >14.5 h, reproductive adults remain reproductive, and at 14.5 h of light, reproductive adults switch slowly to diapause.

**Seasonal Timing of Diapause.** Adult beetles were sampled and scored for developmental status at Lovell in 2002, Pueblo in 2003, and Tinemaha in 2001 and 2002 (Fig. 3), as well as Lovelock and Walker in 2004



**Fig. 2.** Diapause induction in reproductive adults switched to shorter daylengths. Insects were raised from egg to adult at 16:8 (L:D) h, and they were paired and remained under these conditions until all pairs were laying eggs. They were transferred to the indicated photoperiods and were monitored daily for oviposition. Each group started with 30 pairs, and all groups had at least 25 living pairs at the end of the experiment. Temperature was 25°C throughout the experiment.



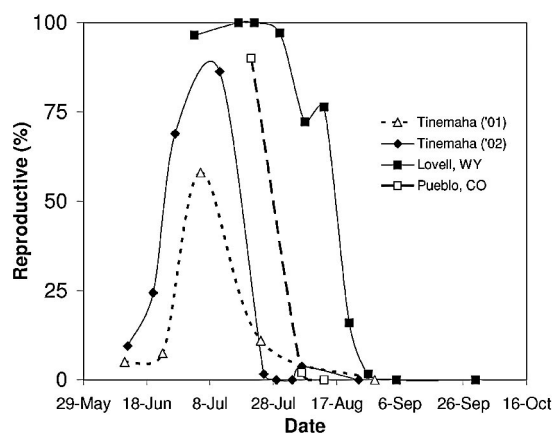


Fig. 3. Seasonal diapause incidence at three field sites. The Tinemaha site in the Owens Valley, CA, sampled during 2 different yr. Samples were 40–80 beetles for most points and included both males and females for Tinemaha 2002 samples and the Lovell samples. Reproductive status was determined by dissection.

(Fig. 4). The summer dates for 50% diapause differed between the five field sites, with the latest date at the most northern latitude and the earliest at the most southern latitude (Table 2). At the Tinemaha site, the majority of insects emerging in late spring entered diapause in both 2001 and 2002 (Fig. 3). At the Walker River site, >30% of the newly emerged beetles collected in late spring also entered diapause (Fig. 4). At the other three field sites, the first summer generation emerges in the early summer rather than late spring (Lewis et al. 2003), and at these sites, early season diapause was not found.

Daylengths when 50% of the population entered diapause ranged from 14 h 04 min at the Lovell site to 14 h 26 min at the Tinemaha site for summer diapause (Table 2). For spring diapause, the daylengths were greater; 14 h 51 min at the Walker site and 14 h 43 min at the Tinemaha site. From these data, the average critical daylength for summer diapause induction was calculated at 14 h 18 min, and for spring diapause, it is 14 h 47 min. These numbers are somewhat lower than the laboratory-based critical daylength measurements, and there are a number of possible reasons for this discrepancy (Tauber et al. 1986). One likely possibility is that, by the time diapause becomes apparent, insects have already responded to diapause-inducing environmental cues perceived in the days preceding entry into diapause. The delay between perception of induction cues and the visible expression of diapause physiology might be long enough to allow significant photoperiod changes in the field. This means that daylength on the day that diapause was first apparent could be quite different from the daylength that induced diapause.

It is difficult to know when diapause is triggered under the ever-changing environmental conditions experienced in the field, but laboratory experiments allow some insight. Even under dramatic shifts in pho-

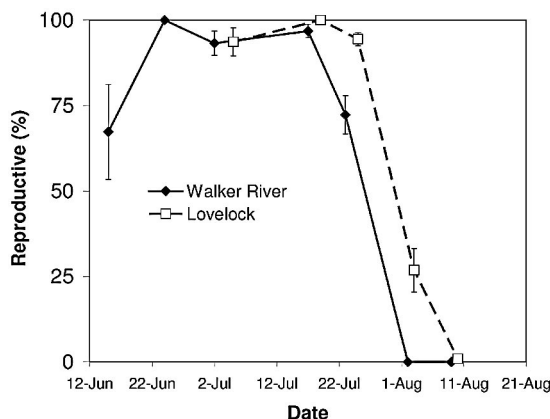


Fig. 4. Seasonal diapause incidence at the Walker (38°53' N) and Lovelock (40°02' N) field sites in 2004. Each point is the average of three to six groups of at least 20 adults.

toperiod, at least 5 d are required to change adults from a reproductive to a diapause program (Fig. 2), whereas under less dramatic shifts, such as to 14.5 h of light from 16 h of light, 20 d are required to shut down oviposition (Fig. 2). These data suggest that it will take at least 5 d and possibly >20 d between the perception of diapause-inducing cues and the expression of diapause physiological characteristics such as the cessation of oviposition. By the time diapause is expressed, daylengths may have changed dramatically and that change will be dependent on time of year and latitude (Fig. 5). When field daylengths are plotted, going back 20 d from the date of 50% diapause induction, they converged 13 d before the 50% diapause date at all field sites and for both summer and spring diapause (Fig. 6). The average daylength at that time was 14 h 39 min, whereas the shortest daylength of the group was 3 min less than the average, and the longest was only 2 min greater.

When a field critical daylength of 14 h 39 min is used to predict 50% diapause dates, they come remarkably close to those observed at all five field sites and including spring diapause (Table 3). These predictions were made by going forward 13 d from the date when daylength reaches 14 h 39 min, determined using sunrise tables (U.S. Naval Observatory 2005), and within this data set, the daylength of 14 h 39 min is a remarkably accurate predictor of field diapause induction.

**Predicting Diapause Induction Based on Field Critical Daylength.** Daylengths never reach 14 h 39 min at latitudes south of 36°20' N (Fig. 5), where most of the first summer generation will enter diapause without reproducing and beetles will be univoltine. For instance, at Temple, the longest days are 14 h 10 min (Fig. 5) and so the first summer generation will fail to reproduce, and the population will be univoltine. North of 36°20' N, daylengths reach 14 h 39 min twice during the season; once in the spring before summer solstice and once in the summer, after summer solstice. As a result there is a seasonal window of potential reproductive activity whose width is a function of

Table 2. The 50% diapause date at five field sites, including two dates at sites where spring and summer diapause were observed

Location (N latitude)	50% diapause (95% CI) <sup>a</sup>	Daylength <sup>b</sup> (50% diapause)
Tinemaha (37°05') spring	June 24 (June 22, June 25)	14 h 43 min
Tinemaha (37°05') summer	July 16 (July 14, July 17)	14 h 26 min
Pueblo (38°16')	July 26 (July 24, July 28)	14 h 19 min
Walker (38°53') spring	June 10 (June 6, June 12)	14 h 51 min
Walker (38°53') summer	July 24 (July 23, July 25)	14 h 22 min
Lovelock (40°02')	July 31 (July 30, Aug 1)	14 h 17 min
Lovell (44°50')	Aug 15 (Aug 13, Aug 18)	14 h 04 min

<sup>a</sup> The dates were calculated by fitting a logistic regression curve to diapause percentage versus calendar date (Figs. 3 and 4). The dates for 50% diapause in the field are given with 95% CL for the 50% points.  
<sup>b</sup> Daylengths were calculated for the 50% diapause date.

latitude, increasing with increasing latitude (Fig. 7). The reproductive window can be calculated for any latitude and is not centered on summer solstice but is offset by 13 d (Fig. 7), with 13 fewer reproductive d in the spring and 13 d more in the summer. Spring diapause occurs when the first summer generation begins to emerge before the reproductive window is open, as shown for the Tinemaha site (Fig. 7). If the entire first generation emerges before the reproductive window opens, the population will be univoltine, because the summer generation will enter diapause without producing a second summer generation. If part of the summer generation emerges before the window opens, and part emerges after it is open, there will be a partial second generation. This is what occurred at Tinemaha during 3 yr of observations (Lewis et al. 2003, Fig. 3). If the entire first summer generation emerges during the reproductive window, a full second summer generation will be produced, which was the situation at the Lovell site. Seasonal timing of the first summer generation is the result of spring temperatures at the three sites. Springs are, on average, warmer at the Tinemaha site, followed by the Pueblo site and then the Lovell site. For instance, the average temperature reaches 12.5°C, the calculated developmental threshold for *D. elongata* (Lewis et al. 2003), during the first week of April at the Tinemaha site, the

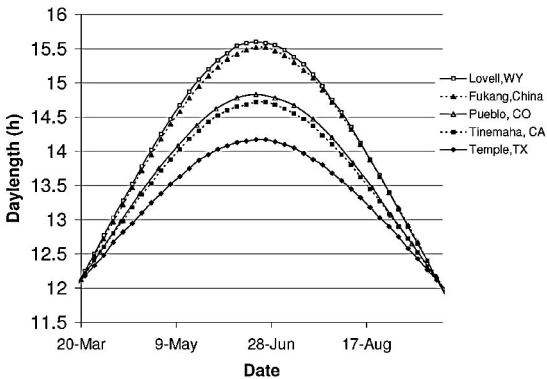


Fig. 5. Daylengths in Fukang (China), Lovell (WY), Pueblo (CO), Tinemaha (CA), and Temple (TX). Information on daylength was obtained from the U.S. Naval Observatory website (<http://aa.usno.navy.mil>), and daylengths were calculated for the year 2003.

first week of May at the Pueblo site, and the second week of May at the Lovell site. For the Pueblo and Lovell sites, the first summer generation emerges after the reproductive window is open, assuring that most of the first summer generation will be reproductive on emergence (Fig. 7), and there will be at least two summer generations. The window of reproductive activity stays open longer into the summer at more northern latitudes (Figs. 5 and 7). For instance, at the Lovell site, the reproductive window is open for nearly 3 wk longer than it is at the Pueblo site, which could have a profound effect on population expansion in the field.

Discussion

The seasonal timing of diapause in *D. elongata* can be predicted based on the photoperiod response in the field. There have been relatively few studies of diapause induction in the field compared with laboratory-based studies, probably owing to the complex array of environmental and physiological factors that may affect the decision to enter diapause under natural conditions. These variables have been outlined and dis-

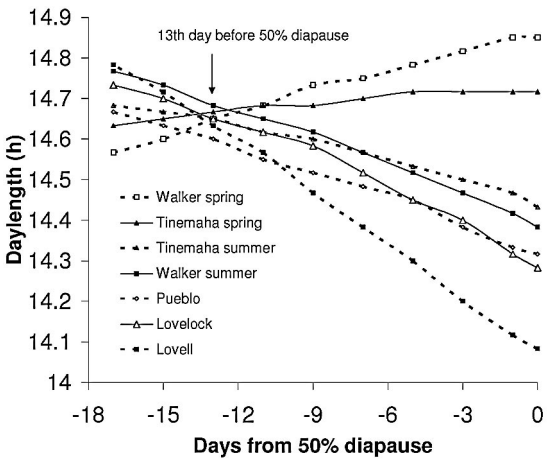


Fig. 6. Daylengths before 50% diapause dates at five field sites, including two dates at sites where spring diapause was detected. Daylengths obtained from the U.S. Naval Observatory website (<http://aa.usno.navy.mil>) were calculated for the year in which each experiment was conducted.

**Table 3.** Daylengths 13 d before the 50% diapause date at five sites, including the spring diapause dates for the Tinemaha and Walker sites

Location (N latitude)	Daylength (50% diapause)	Daylength 13 d before 50% diapause <sup>b</sup>	Measured 50% diapause date	Predicted 50% diapause date <sup>c</sup>
Tinemaha (37°05') spring	14 h 43 min	14 h 40 min	June 24	June 23
Tinemaha summer	14 h 26 min	14 h 39 min	July 16	July 16
Pueblo <sup>a</sup> (38°16')	14 h 19 min	14 h 36 min	July 26	July 24
Walker (38°53') spring	14 h 51 min	14 h 39 min	June 10	June 10
Walker summer	14 h 22 min	14 h 41 min	July 24	July 26
Lovelock <sup>a</sup> (40°02')	14 h 17 min	14 h 39 min	July 31	July 31
Lovell <sup>a</sup> (44°50')	14 h 04 min	14 h 38 min	Aug 15	Aug 14

<sup>a</sup> Only a summer date was used because there was no observed spring diapause at these sites.

<sup>b</sup> The average daylength 13 d before 50% diapause was 14 h 39 min.

<sup>c</sup> The predicted 50% diapause date was calculated by locating 14 h 39 min on daylength charts and advancing 13 d.

cussed by Tauber et al. (1986), and they include the interaction between photoperiod and other factors, primarily temperature and nutritional status, the differential photoperiod sensitivity of life stages, differential responses to daylength above and below the critical daylength, effects of changing daylengths, effects of previous photoperiod exposures, and spectral sensitivity to twilight. It was stated that "as a result, the ability to predict diapause induction and to characterize diapause intensification in field populations lags far behind our knowledge of photoperiodic responses in the laboratory" (Tauber et al. 1986).

Critical daylength for diapause induction is profoundly influenced by temperature in many species (Tauber et al. 1986, Danks 1987, Leather et al. 1993), decreasing by as much as 3 h in response to a 10°C temperature increase (Danilevsky et al. 1970). In some species, diapause may even be eliminated at high temperatures (Chippendale 1982). In contrast, critical daylength for diapause induction in *D. elongata* varied by only a few minutes under a range of temperatures, including a thermoperiod. In some species, host plant or host plant condition can influence the decision to enter diapause (Hsiao 1978, Hare 1983), but in *D. elongata*, we have not seen this, even though we reared the insects on two different species of *Tamarix* and have used both live plants and cut bouquets for rearing.

Environmental conditions experienced during previous life stages or even the previous generation may influence diapause induction in many species. In *D. elongata*, diapause induction can occur even in reproductive adults, and the critical daylength for switching reproductive adults to diapause is nearly the same as that measured for insects reared from eggs under a single light regimen. This response, known as indeterminate diapause (Taylor and Spaulding 1988), gives *D. elongata* maximum developmental flexibility with respect to diapause. The entire population, regardless of age or previous reproductive status, may enter diapause nearly simultaneously in the field with previously reproductive adults lagging slightly behind newly emerged adults.

In many insect species, some period of twilight is measured as day light so that the exact length of a day in the field is difficult to determine (Beck 1980). In *D. elongata*, there is close agreement between critical

daylength measured in the laboratory (14 h 53 min under a thermoperiod) and that measured in the field (14 h 39 min), which indicates that perhaps ≈14 min of twilight time is perceived as daylight. If only 7 min of twilight are added at dawn and dusk to the perceived daylength, the critical daylengths in the laboratory and field would be equal. Civil twilight at 40° N latitude is over 1 h during the early days of summer (Beck 1980), so *D. elongata* apparently perceives <25% of the time of civil twilight as daylight.

In conclusion, diapause in *D. elongata* is exceptional in that photoperiod is the primary determinant, with other factors playing little or no role. This allows the use of photoperiod to accurately predict the developmental status of populations in the field.

**Photoperiod, Diapause Induction, and Phenology of *D. elongata*.** The overwintering generation of *D. elongata* emerges from the leaf litter and begins feeding as soon as *Tamarix* foliage becomes available in the spring (Lewis et al. 2003). Reproductive development begins immediately, requiring no specific photoperiodic stimulation (D.B., unpublished data), the same as with many insect species (Tauber and Tauber 1970, Tauber et al. 1986). The progeny of the overwintering generation become first-generation adults in the late spring or early summer, depending on the location (Lewis et al. 2003). The developmental fate of the first and any subsequent summer generations is photoperiod dependent.

At latitudes south of 36°20' N, daylengths never reach the field critical daylength of 14 h 39 min, so *D. elongata* will be essentially univoltine with the first generation entering diapause to become the overwintering generation. At latitudes north of 36°20' N, a seasonal window of reproductive activity may allow a second (Lewis et al. 2003) or even a third generation at more northern sites (Li et al. 2000). After daylengths fall below 14 h 39 min, there is a 13-d lag period, and newly emerging adults enter diapause without becoming reproductive, whereas reproductive adults begin to switch to diapause.

Daylengths at the time of entry into diapause vary greatly (Table 2) depending on time of year and latitude, but 13 d before entry into diapause, daylengths show dramatic convergence on 14 h 39 min (Fig. 6). There are three properties of seasonal photoperiod change that account for this convergence and the near

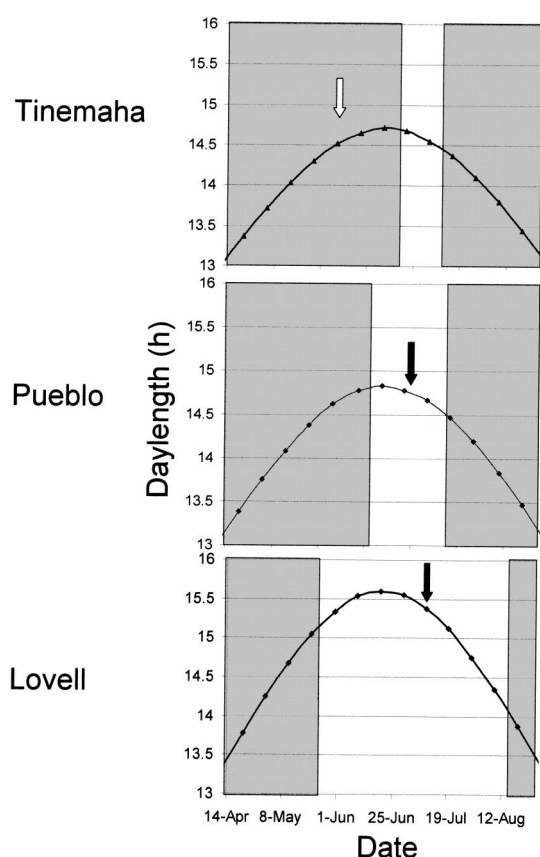


Fig. 7. Predicted seasonal reproductive activity for *D. elongata* at three field sites. White area indicates the dates when beetle populations are >50% reproductive and the gray areas when they are <50% reproductive. Block arrows indicate when the first summer generation emerges at each site (Lewis et al. 2003, D.B., unpublished data).

elimination of daylength differences 13 d before the 50% diapause date across the range of field sites. First, spring daylengths increase until summer solstice, so 13 d before spring diapause, the daylengths are shorter, which decreases values for the two longest daylengths shown in Table 2. Second, the rate of daylength change increases as the summer progresses, so going back 13 d in the late summer adds more daylength than it does earlier in the summer. Finally, rates of daylength change are greater at higher latitudes, so going back 13 d at a site such as Lovell will add more daylength than at a more southern site such as Tinemaha (Fig. 5). As a result of these geophysical-based properties of seasonal photoperiod curves (Fig. 5), the longest field critical daylengths, recorded in the spring, are shortened by going back in time 13 d. In addition, the shortest field critical daylengths increased the most by going back 13 d, because they were from the most northern sites and occurred later into the summer.

Because critical daylength for diapause induction in reproductive adults is 15–30 min shorter than critical

daylength for diapause induction in prereproductive insects (Table 1; Fig. 2), it is expected that reproductive adults will continue to be reproductive, even as newly emerged adults are entering diapause. As daylengths decline further, reproductive adults also enter diapause. This pattern has been observed in the field; when the newly emerged adult population begins to show a high percentage of diapause individuals, the older reproductive adults continue to oviposit and then they too enter diapause (D.B., unpublished data). After a period of feeding, all diapause insects descend from the host plants and enter the leaf litter beneath the plants until the following spring (Lewis et al. 2003, Bean et al. 2007).

First-generation adults may emerge before the opening of the reproductive window resulting in a spring diapause, as was noted at the Tinemaha and Walker sites (Figs. 3 and 4). For the first summer generation to realize full reproductive potential, the window must be wide enough and must be open by the time the first summer generation emerges as adults. Higher spring temperatures, which are correlated with lower latitudes, will position the first generation earlier in the season and increase the risk that the first generation will emerge before the reproductive window. This means that there are two factors working to place the first summer generation outside of the reproductive window at southern latitudes: earlier emergence of adults in the first generation and a narrower reproductive window.

Another consequence of latitude-dependent reproductive activity is a lack of synchrony between beetle populations and the host plant at more southern latitudes. Southern latitudes generally have longer growing seasons and extended host plant availability, yet the beetles have fewer days of reproductive activity in which to take advantage of those resources. Synchrony with resources would be optimized if diapause were induced later in the year at more southern latitudes (Tauber et al. 1986, Masaki 1999), but this is the opposite of what happens in the field.

**Properties of Diapause Limit Effective Range of *D. elongata*.** Being univoltine does not preclude success as a biocontrol agent (Tauber et al. 1996), but slower growing populations mean that it will take beetles longer to reach densities where impact on tamarisk can be measured, clearly a detriment to the biological control program.

Diapause destined insects express behavior patterns that are adaptive for winter survival (Bean et al. 2007), but not for the population expansion that would best suit biological control. Diapause associated behaviors include a decrease in adult feeding, which means less harm to the target plants, a decrease in dispersal behaviors, which means less chance of population spread, and eventual movement into the leaf litter where insects cannot damage the plant. From an ecological point of view, early entry into diapause allows a substantial period of time for host plants to refoliate and possibly compensate for impacts experienced earlier (Dudley and DeLoach 2004). Late season recov-



ery of the host plant would severely restrict efficacy of the biocontrol agent.

Early diapause will slow population growth, and smaller populations will be more vulnerable to extinction (Allee effects) than would be larger, rapidly expanding populations. Ant predation (Herrera 2003) seems to play a role in inhibiting establishment, but large rapidly growing populations are able to overcome ant predation (Dudley and DeLoach 2004). A number of other observations indicate that the success of *D. elongata* as a biocontrol agent depends on aggregation behaviors allowing beetles to achieve high densities. At successful test sites, the beetles defoliate large portions of plants, entire plants, or even whole stands of tamarisk when populations are thriving (DeLoach et al. 2004, Dudley and DeLoach 2004). In the field, we have observed hundreds, even thousands of reproductive adults massing in the tops of tamarisk plants, with the subsequent production of progeny that defoliate the entire plant (unpublished data). These aggregations of reproductive adults seem to be part of the biology of this species, brought about in part by the release of a male-produced aggregation pheromone blend (Cossé et al. 2005). Overwintering *D. elongata* also form aggregations in the leaf litter, ranging from a few individuals to several hundred (unpublished data), which may benefit over winter survival, as shown in other beetle species (Tanaka et al. 1988, Yoder et al. 1992). Small, slowly growing populations of *D. elongata* would not be able to easily achieve large aggregations, making them more vulnerable to extinction.

In their native range, beetles enter hibernial (overwintering) diapause in the late summer, when temperatures are declining, but in advance of freezing temperatures (Li et al. 2000). In *D. elongata*, diapause is a behavioral and physiological adaptation for winter survival, not for long-term survival during hot, dry summer conditions experienced in the western United States. In laboratory trials, we found that survival rates for diapausing beetles decline dramatically at increasing temperatures from 5 to 25°C (data not shown), which is probably caused by an increased metabolic rate and faster use of metabolic reserves at higher temperatures (Thompson and Davis 1981). By the time winter comes to lower latitude sites, such as Tinemaha, where many of the beetles entered diapause in mid-June, metabolic reserves will be seriously compromised, because beetles will have spent 3 mo in diapause at temperatures averaging >25°C. In addition, ants and other predators are active in the leaf litter during the summer months, making it a dangerous place for dormant beetles. It is likely that most of the adults that entered a premature diapause in June or early July will be dead or compromised by low metabolic reserves by the time that low temperatures come in late September.

Premature diapause will slow population expansion, may lead to extinction of introduced populations, will be detrimental to synchrony with the host plant, and will compromise over winter survival, but it is difficult to precisely define a latitudinal cut off for success of

*D. elongata* in North America. Below the 36th parallel, populations will be primarily univoltine, and whereas there were not many introductions south of the 36th parallel, those that occurred were not successful. Just north of the 38th parallel, populations may achieve nearly 100% reproductive activity for a short time in the early summer. Success at two sites, the Pueblo site at 38°16' N and the Walker site at 38°53' N, is evidence that this population can be effective near the 38th parallel. At latitudes between 36 and 38° N, there is potential for a partial second generation and perhaps success at some locations, but there are several factors working against effective biocontrol in these areas. Spring diapause, early summer diapause, and the increased time for tamarisk regrowth after entry into diapause make it unlikely that the Fukang population will succeed as a biocontrol agent south of the 38th parallel. The Tinemaha site (37°05' N) is a good example of what can be expected of population performance at the intermediate latitudes. Beetles reproduce in the early summer, but the nature and timing of diapause allow only a small second generation, and early entry into diapause probably decreases overwinter survival. Tamarisk biocontrol using *D. elongata* failed at the Tinemaha site and has been suspended there indefinitely. In conclusion, we do not recommend *D. elongata* originating from Fukang, China, for tamarisk biological control south of the 38th parallel.

**Other Populations May Be Useful at Southern Latitudes.** The critical daylength for diapause induction limits this population of *D. elongata* to latitudes similar to the latitude of origin, and this limitation may persist for years under field conditions (Goldson and Emberston 1980), although critical daylength will be under heavy selection pressure to evolve toward an optimal value for resource use (Taylor and Spaulding 1988). Evolution of critical daylength will certainly occur within established populations, but the rate of such evolution may not be fast enough for the practical constraints of the tamarisk biological control program. This is especially true for the initial introductions of this agent. These introductions are typically small, consisting of a few thousand adults, and these small populations may be particularly vulnerable to extinction before natural selection can come into play. For this reason, testing other populations of *D. elongata* for shorter critical daylengths is essential.

Fortunately, like the host plant genus (Baum 1967), the native range of *D. elongata* is vast and includes nearly every conceivable temperate and subtropical climate type. Collections of this taxon have been made from 15° N latitude to above 45° N latitude on three continents (DeLoach et al. 2004). This represents a wealth of genetic diversity and numerous options for matching populations with geographic regions in the western United States. In other species, the correlation between latitude of origin and critical daylength has been well documented (reviewed in Danilevsky et al. 1970, Beck 1980, Tauber et al. 1986, Danks 1987, Leather et al. 1993), and in some species, critical daylength for diapause induction can be accurately predicted based on latitude of population origin (Brad-

shaw 1976, Masaki 1999). It is likely that other populations of *D. elongata* will have appropriate photoperiod responses as well as other potentially important physiological characteristics to enable them to thrive in areas where the Fukang population has failed (DeLoach et al. 2004).

### Acknowledgments

The authors thank D. Kazmer (USDA-ARS NPARL, Sidney, MT) and D. Eberts (USBR, Denver, CO) for collections from the Lovell and Pueblo field sites; R. Bartelt (USDA-ARS NCAUR, Peoria, IL) for helpful comments on the manuscript; and T. Wang, D. Laclergue, and M. Ware for technical assistance.

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*Received for publication 10 July 2006; accepted 25 October 2006.*

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